A Chloride and Oxygen Analysis Kit for Pond Waters[1]

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INTRODUCTION

FISHPONDS IN THE HAWAIIAN ISLANDS are shallow brackish ponds which are frequently fed by underwater springs as well as surface flows of fresh water and sea water. The chloride content of the water is an indication of the quantity of sea water present and is one factor which affects the growth and survival of many of the plants and animals utilized as food by the fish. The presence of dissolved oxygen is absolutely essential for animal life in the ponds. Low oxygen concentrations in some regions indicate stagnant waters and bottom putrefaction which make these areas unfit for fish. In the course of fisheries studies in the Hawaiian Islands it was therefore necessary to make a large number of salinity and oxygen determinations on fishpond waters.

Since both chloride and oxygen may vary severalfold in a single pond, and since most living organisms are insensitive to concentration changes which are less than 1 per cent of the normal value, it is not necessary to make extremely accurate determinations. Methods were desired for the rapid analysis of chloride and oxygen at the side of the pond so that any unusual observations could be checked before one left the pond.

The apparatus described in this paper was designed to provide sufficient equipment and reagents for 25 chloride, and 50 dissolved oxygen determinations. Two auxiliary half-liter bottles contain enough reagents for an additional 50 chloride determinations. Chloride up to 25 gm. per liter (sea water is about 20 gm. per liter) can be determined with an accuracy of 0.06 gm. of Cl per liter. Dissolved oxygen up to 20 cc. per liter can be determined with an accuracy of 0.1 cc. per liter on a sample of 10 cc. of water. The apparatus weighs about 25 lb. and can be used in a rowboat, if necessary. A single chloride determination requires about 3 minutes; an oxygen determination requires about 8 minutes.

Although there are several well-known titrimetric methods for chlorides, none of them is suitable for use on micro-samples out-of-doors where it is difficult to observe a colorimetric end point. An electrometric method of detecting the end point which substituted a galvanometer needle for the color change appeared to be more suitable for our purposes. None of the published electrometric methods was quite satisfactory and an entirely new method was developed which will be dealt with in another publication (Dean and Hawley: unpublished). This method makes use of two wire electrodes, a galvanometer, a few radio resistances, and a dry cell. A relatively low resistance circuit is formed which is not sensitive to high humidities, although it should not be soaked in water.

Krogh (1935: 131–133) has described a modification of the Winkler method for dissolved oxygen which was well suited to our purpose, with slight modifications. Krogh used the conventional starch indicator to detect the end point. We have substituted

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Foulke's dead-stop electrometric end point (Foulke and Bawden, 1926: 2045 ff.), because the starch iodine indicator is unreliable above 25° C. and our field temperatures frequently exceed 30° C. Essentially the same electrical circuit is used for both the oxygen and the chloride end points: only the electrodes are different.

The unknown solutions are titrated with standard solutions from a micrometer syringe burette (see Trevor, 1925: 1111; Dean and Fetcher, 1942: 237; and Dean: unpublished). This burette has many advantages over conventional gravity-feed burettes. It is more compact, there is no drop error and no parallax error, and better than 0.1 per cent accuracy is possible on a total volume of only 1 cc. of reagent. Micro methods are obviously necessary to conserve reagents if a small apparatus is to carry enough to permit 50 or more determinations. Two syringes are used interchangeably with the same micrometer head in this apparatus, so that it is not necessary to clean and refill the burette when changing from chlorine to oxygen analyses.

DESCRIPTION OF THE APPARATUS

All of the equipment is contained in a wooden box which measures $9 \times 9 \times 12$ inches (see Fig. 1). The electrical wiring is enclosed in a mahogany case which is firmly attached to the right-hand side of the box. The galvanometer is a needle type of instrument which has a sensitivity of 0.25 microamperes per scale division and a critical damping resistance of 1,800 ohms.

The electrical circuit is shown schematically in Figure 2. The two switches S_1 and S_2 are combined in a telephone type of toggle switch. In the central position both switches are open and no current flows. When the switch is moved to either side, current flows from the battery through the series of resistors in the upper line. Suitable taps take off about 10,200 and 1,018 mv. to the Pt, Ag, and Standard Cell terminals respectively. Variations in the internal resistance of the dry cell can be compensated for by adjusting resistor R_2 when the switch is thrown to the left. In this position the voltage of the standard cell opposes a fraction of the voltage from the battery. Resistor B is adjusted until no current flows through the galvanometer. In actual practice it has been found that the voltage from a flashlight dry cell does not change significantly, even when the circuit is left open for 2 days. It would have been satisfactory to omit the standard cell and variable resistor B entirely. The potential at the chloride electrodes could be checked before each series of measurements, as is described below.

The electrodes are constructed as shown in the insert of Figure 2. Short pieces of silver or platinum wire are attached to stiff bronze wire with hard solder. The wire electrode is then sealed in a straight glass tube. It is not difficult to seal platinum into soft glass. Silver wire of 22 gage can also be sealed into soft glass but some of the seals break soon after sealing. After the glass is sealed onto the electrode wire, both the glass tubing and the bronze wire can be bent in the ordinary manner, since bronze wire softens at red heat.

The stiff bronze wire is passed through a hole drilled in a small radio jack plug as indicated in the diagram. The four electrode leads—Ag, Cu, and two Pt—are brought to jacks on a mounting plate at the left end of the box under the end of the burette. When either pair of electrodes is plugged into its corresponding jacks, the electrode wires just reach the center of the titration vessel. The Ag and Cu jacks are further differentiated by color to reduce the danger of inserting an electrode in the wrong terminal.

The micrometer-syringe burette consists of a 1.5 cc. glass hypodermic syringe fitted in a frame to which is attached a 1-inch machinist's micrometer graduated in 1,000 parts. The burette assembly is described fur-

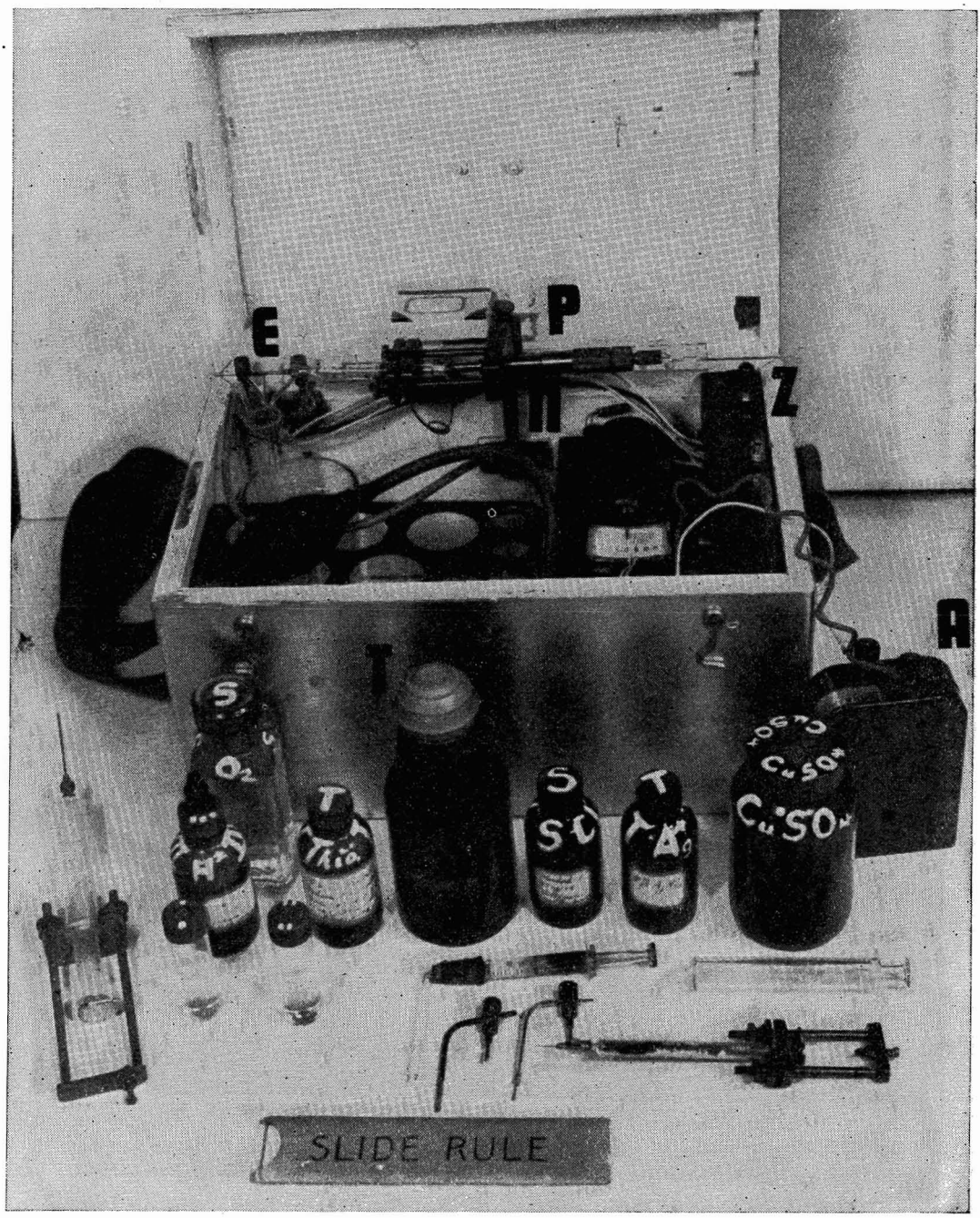

FIG. 1. Analysis kit for chloride and oxygen. Chloride reagents and syringes to the right; oxygen reagents and syringe to the left. *E*, electrode holder. The chloride electrodes are just above the slide rule. *P*, micrometer burette with syringe to hold sodium thiosulfate reagent. The silver nitrate syringe is above chloride electrodes. *N*, galvanometer. *Z*, toggle switch. *A*, standard cell. *T*, rubber bulb in bottle of distilled water for washing. The syringes and slide rule fit in clips inside the lid of the box. Most of the equipment remains in the box during the analysis.

ther in Dean and Fetcher (1942: 237; see also Dean: unpublished). For the oxygen determination a solution of thiosulfate is used in the burette and a 2-inch 22-gage hypodermic needle bent downward at 90° serves as the delivery tip. The chloride determination requires a strong solution of silver nitrate, which corrodes all available metals, so that a glass tip must be used. In this apparatus we used a bent glass tube attached to the burette by a number 0 one-hole rubber stopper.[3]

[3] The Ace Glass Company, Vineland, N. J., is now able to supply ground glass tips to order which will fit on a standard hypodermic syringe.

The titration vessel is a shortened test tube about 25 mm. in diameter and 70 mm. long. It fits in a wire test tube holder which is attached to the electrode jack assembly. The burette tip and the two electrodes dip into the solution in the titration vessel. In addition there is a glass tube to introduce air bubbles for stirring. Air is forced by an aspirator bulb into a 1-liter can which serves as an air reservoir, and flows from there through a thin rubber tube to the glass nozzle of the air stirrer. The aspirator bulb can be conveniently squeezed by the left hand of the operator during a titration.

A reagent shelf over the air reservoir

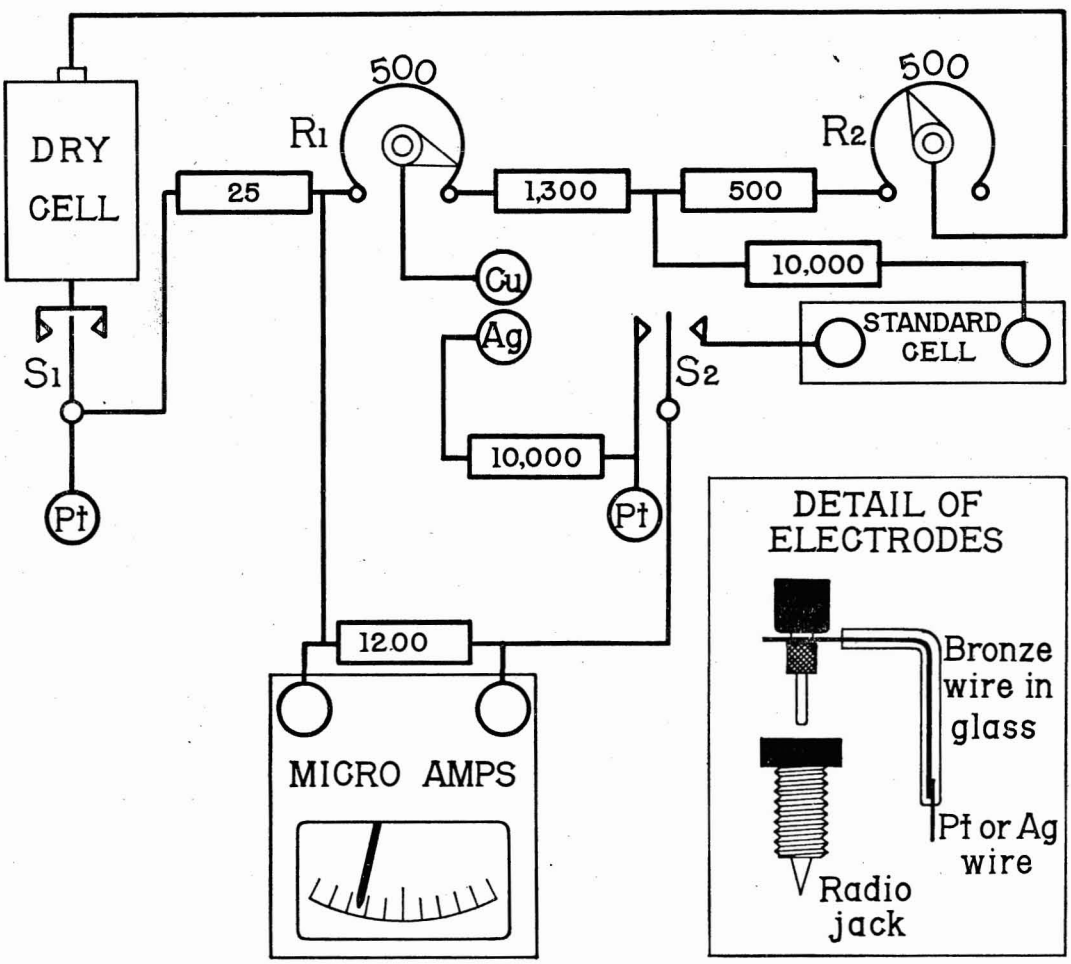

FIG. 2. Wiring diagram. S_1 and S_2 are two parts of a double-pole, double-throw telephone-type toggle switch. Wires run from the Pt, Ag, and Cu terminals to the electrode holder.

holds five 60-cc. screw top bottles and two 10-cc. screw top vials. In addition, there are several larger reagent bottles in the bottom of the box and a bottle of distilled water for washing electrodes and glassware. A glass tumbler is fitted under the electrodes for a waste jar. A rubber ear syringe is fitted with a glass delivery tip bent at an angle of 135°. This syringe can be filled from the distilled water bottle and the water in it then directed in a stream into the inverted titration vessel or onto the electrodes to clean them.

The reagents are all contained in screw cap bottles or vials. The screw caps are lined with Polythene, which is resistant to all the reagents used in this work. Two of the vials are fitted with rubber disks from 10-ml. penicillin bottles. The disks are held on by screw caps which have been drilled with 1/4-inch holes. The rubber disks on the vials can be repeatedly perforated by a hypodermic needle and the reagents withdrawn through the needle.

All the reagents and equipment used for the chloride analysis are marked with black paint and the various reagents are identified by white letters on the top, as well as by conventional labels on the sides of the bottles. The reagents and apparatus for the oxygen determination are likewise marked with red paint and white letters. The metal parts of the syringe pipettes and the burette are of brass which was first dulled by a dip in silver nitrate and nitric acid, and then coated with Glyptal varnish which was baked on. This treatment produces a non-glaring, corrosion-resistant surface. The syringes are attached by clips to the inside of the top of the box. A small box of filter paper squares, about 3 cm. on a side, which are used to wipe the burette tips and the electrodes, is also attached inside the top of the box. A rubber stopper is screwed inside the top at the right-hand side in such a way that the lid will not close until the switch has been turned off. This arrangement insures that the battery will not be left on when the box is closed.

CHLORIDE ANALYSES

The chloride analysis depends upon the fact that the potential of a silver electrode changes rapidly when all the chloride ions have been precipitated by silver ions. Since it is impossible to measure a single electrode potential, another electrode which is not sensitive to chloride ions must be present to complete the circuit. We have used a copper electrode in the presence of nearly saturated copper sulfate. The potential between these two electrodes is about 200 mv. at the end point. The potentiometer circuit supplies 200 mv. between the Cu and Ag electrodes and, at the end point, no current will flow through the galvanometer.

Before the end point is reached the potential between the electrodes will be less than 200 mv. and the galvanometer will be deflected to the right. There is a large protective resistance in series with the chloride electrodes so that the galvanometer may be left continuously in circuit. As the end point is approached the galvanometer needle approaches zero and is deflected to the left as soon as the end point has been exceeded. The deflection produced by the addition of one or two burette divisions of silver nitrate is greatest at the end point. This fact can be used to set the potentiometer to the correct potential. The potentiometer is adjusted until the galvanometer indicates zero. The galvanometer deflection is then noted after the addition of two burette units of silver nitrate. This procedure is repeated until the galvanometer deflection is a maximum. The potentiometer is left at this setting for subsequent titrations.

The silver nitrate reagent contains 4 per cent silver nitrate and 0.5 per cent nitric acid. The burette is filled by bringing the reagent bottle of silver nitrate, marked with a T on a black screw cap, up under the burette tip. The plunger of the syringe is withdrawn by unscrewing the micrometer, and silver nitrate is sucked into the syringe.

Any air bubbles are displaced by rotating the burette in its clamp until the tip is upwards and then forcing a little of the silver nitrate reagent out of the tip. The burette is filled up to the 1,000 mark on the micrometer and the tip rinsed with water.

Ten cc. of saturated copper sulfate is placed in the titration vessel; a syringe with tip broken off is used to make the transfer. Commercial bluestone can be used for this solution, although it may introduce an undesirably high blank. Reagent grade copper sulfate is perfectly satisfactory. The copper sulfate solution is placed under the electrodes and the air stirrer is started. A small residual concentration of chloride ions may produce a galvanometer deflection to the right. If it does, silver nitrate is added until the galvanometer reads zero. The burette reading is recorded. The chloride sample, about 0.4 cc., is then introduced from a precision syringe pipette (Krogh, 1935: 130; Dean: unpublished). Silver nitrate is added until the galvanometer again indicates zero and the second burette reading is recorded.

Neither the exact volume relations of the syringes nor the concentration of the silver nitrate need be known exactly. All these are evaluated as a single factor by titrating the same volume of a known standard solution of sodium chloride which contains exactly 20 gm. of chloride ions per liter.

When the galvanometer has been brought to zero a second time and the burette reading has been recorded, the air stirrer is removed and wiped. The burette is tipped up and the tip is wiped and the burette is refilled. The electrodes and the burette tip are rinsed and the apparatus is ready for the next determination.

The errors are of the order of 1 burette division or 1 part in 800. Greater precision could be obtained by precipitating most of the chloride in a larger sample with silver nitrate from another pipette. A more dilute solution of silver nitrate could then be used in the burette. It might be advisable to carry out such determinations in more dilute solutions to avoid too much clumping of the silver chloride precipitate. The accuracy is limited by the reproducibility of the syringe pipettes. Krogh (1935: 130) reports an accuracy of 1 part in 10,000 and we have several syringes with an accuracy of 2 parts in 10,000.

OXYGEN ANALYSES

Oxygen is determined by its reaction with a solution of manganous hydroxide to form manganic oxides. When all the oxygen has been absorbed, the solution is made acid and the manganic ions react with iodide ions to liberate free iodine. The iodine is titrated with sodium thiosulfate in the presence of two bright platinum electrodes. A potential of 10 mv. is applied between these electrodes and a current will flow as long as there is iodine in the solution to remove electrons from the negative electrode. As soon as all the iodine has been removed the current ceases to flow, except for a very small residual current. As the end point is approached, the galvanometer deflection decreases from the left and the end point is taken when the galvanometer indicates one unit deflection to the left.

A 10-cc. syringe pipette (Krogh, 1935: 132; Dean: unpublished) is fitted with a 2-inch 18-gage stainless steel needle. An aluminum cam cemented to the plunger with Varno cement will engage a stop on the side of the syringe holder when the syringe holds 10 cc. An additional 0.2 cc. can be introduced by rotating the cam away from the side stop and pulling back until the plunger reaches an end stop.

The syringe is first rinsed with Solution I. This solution is made up by dissolving 90 gm. of NaI and 40 gm. of NaOH in 55 cc. of water (Pomeroy and Kirschman, 1945: 716) and all air bubbles are expelled. This

reagent is kept in a small rubber-capped vial marked on each side with one white dot. The needle of the syringe is inserted into the vial and the vial is inverted while the solution is drawn in and the air bubbles are expelled. This leaves about 0.1 ml. of solution in the dead space of the syringe. The water sample is then drawn into the syringe until the cam on the plunger reaches the side stop. The needle is then inserted into a second vial, marked with two white dots, which contains Solution II. This solution is made from 40 gm. of $MnCl_2$, 10 cc. of 6N HCl, and enough water to make 100 cc. This solution is drawn into the syringe by moving the plunger from the side to the end stop.

The manganous chloride reacts inside the syringe with the sodium hydroxide which was left in the dead space, to produce a light fluffy precipitate of manganous hydroxide. This precipitate absorbs oxygen, and in 4 minutes substantially all of the oxygen will have been absorbed. The contents of the syringe are then discharged below the surface of 1 cc. of 6N HCl in the titration vessel. The syringe is rinsed first with the acid solution to dissolve any manganous hydroxide remaining in the syringe, and then with two small portions of water from a spare titration vessel. The 6N HCl is contained in a 60-cc. bottle fitted with a rubber medicine dropper. One dropper full is about 1 cc.

The titration vessel now contains iodine equivalent to the oxygen which was present in the water. The iodine is titrated with a sodium thiosulfate solution. This solution must be made up fresh every week by diluting to 60 cc. one medicine dropper full of a 60 per cent solution of sodium thiosulfate containing 1 per cent borax. This concentrated solution is quite stable. Most of the iodine is discharged with thiosulfate from the burette before the air stirrer is started, because the air might otherwise remove some of the iodine.

The thiosulfate solution and the volumetric apparatus are standardized by the use of a standard solution of KIO_3. This solution is 0.0004167 molar and is equivalent to 14 cc. (STP) of oxygen per liter. The dead space of the syringe is first filled with Solution I. The syringe is then rinsed with acid contained in the titration vessel and filled with the standard iodate solution. The iodate reacts with the iodide and acid to liberate iodine in the syringe. The iodine is titrated with thiosulfate, and the burette difference which is found corresponds to 14 cc. of oxygen per liter. Blank runs on water which had been freed of oxygen by hydrogen and platinized asbestos showed that the end point and other errors are less than 0.1 cc. of oxygen per liter.

The oxygen concentration is calculated in essentially the same manner as that used for the chloride. The initial burette reading is always taken as 1,000. This procedure introduces a small constant error of the order of magnitude of 0.1 cc. of dissolved oxygen per liter.

SUMMARY

A portable apparatus which is equipped for the determination of chlorides and dissolved oxygen in pond waters is described. The chloride is determined by a new electrometric method. Oxygen is determined by a modified Winkler method and the iodimetric end point is detected electrometrically. The volumetric apparatus consists of precision syringe pipettes and a micrometer burette. Up to 25 gm. of Cl per liter can be determined with an accuracy of 0.06 gm. per liter. Up to 20 cc. of dissolved oxygen per liter can be determined with an accuracy of 0.1 cc. per liter. Greater precision over a smaller range of chloride concentration is possible, since the syringe pipettes can attain an absolute accuracy of one part in 10,000.

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